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DEHYDRATION AND SHOCK: AN ANIMAL MODEL OF HEMORRHAGE AND RESUSCITATION OF BATTLEFIELD INJURY.

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ABSTRACT: We have developed a porcine model of the anticipated military use of oxygen-carrying resuscitation solutions. The objective is to determine whether toxicity under adverse conditions will limit further development of hemoglobin-based products. Splenectomized immature female swine are used because of their extensive use in the evaluation of other resuscitation solutions. Five days prior to each experiment, central vascular catheters and a renal arterial flow probe are surgically placed in the animals. After recovery and weight gain has resumed, animals are placed in metabolic cages and deprived of water for 48 hours to produce hyperosmolar dehydration resulting in loss of approximately 7% of body weight. We remove 38% of estimated blood volume, 25 ml/kg, over one hour by a controlled logarithmic hemorrhage. Resuscitation is by administration of a fixed volume of test solution. Hemodynamic function is observed but no further therapy is given for three hours, a period corresponding to evacuation in the field. After this period, corresponding to arrival at a field hospital, the animals' blood is returned. Swine are then observed in metabolic cages for an additional 7 days while blood and urine are sampled daily. At the end of this period, animals are anesthetized, urinary catheters are implanted, and creatinine clearances are measured. Swine are than euthanized, and their tissues are examined. In a pilot study, resuscitation was performed with either Ringer's lactate, albumin, stroma-free hemoglobin, or cross-linked ($\alpha\alpha$Hb) hemoglobin. All animals survived.

INTRODUCTION: In the Spring of 1991 the United States Army Medical Research and Development Command asked the commercial producers of hemoglobin-based oxygen carriers for permission to test their products in an animal model of resuscitation from hemorrhagic shock[1]. As part of the request, the Army circulated a protocol which described such a model using immature Yorkshire swine. The purpose of the testing was to determine the toxicity of hemoglobin- based oxygen carriers under the conditions of anticipated military use. For the Army, the conditions of anticipated field use are a recipient who is significantly dehydrated before going into hemorrhagic shock. These conditions were expected to elicit the historic renal toxicity of hemoglobin solutions.

Swine were used in the protocol because timed water restriction leads to reproducible body water loss, and because swine are a good model of human

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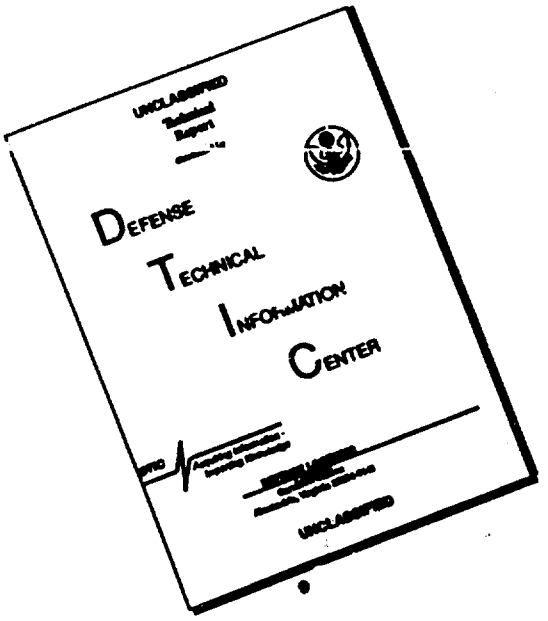

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cardiovascular dynamics. The protocol was subsequently endorsed by an official of the Food and Drug Administration (FDA) at a major transfusion medicine meeting[2], and the rationale of that endorsement was given in a public policy statement on the evaluation of hemoglobin solutions by the Center for Biologics Research and Evaluation of the FDA[3].

This paper describes the design of the Army protocol for testing the safety of hemoglobin-based oxygen carriers. It also describes a pilot study we conducted to verify that the hemorrhage induces shock and that the resuscitation allows the study of the toxicity of hemoglobin solutions.

PROTOCOL: We buy immature female Yorkshire swine weighing 10 to 15 kg from a commercial breeder and observe them in quarantine to document good health and weight gain. Five to eight days before hemorrhage and resuscitation, the swine undergo splenectomy and surgical placement of arterial, central venous, and pulmonary artery catheters and placement of a left renal artery ultrasonic blood flow probe. Catheters and transducer wires are brought through the skin in the dorsal midline and secured in Velcro pouches. Animals are then allowed several days to recover from surgery and reestablish weight gain.

Two days prior to hemorrhage and resuscitation, the animal is transferred to metabolic cages, drinking water is withheld, and daily blood sampling and urine collection are instituted. On the day of hemorrhage and resuscitation, the swine is brought to the laboratory in the metabolic cage and confined to a narrow portion of the cage to limit movement. When the animal lies down, catheters and the flow probe are attached to transducers and a chart recorder. After two resting measures of hemodynamics, oxygen transport, blood cell counts, and blood chemistries 15 minutes apart, hemorrhage of 25 ml/kg (38% of estimated normal blood volume) is conducted in four 15 minute intervals with removed volumes of 10, 7, 5, and 3 ml/kg. Measures and blood samples are taken at the end of each 15 minute interval. Five minutes after the end of the hour-long hemorrhage, the animals are resuscitated by intravenous infusion of a fixed volume of either test or control solution. Measures and blood samples are taken at five time points during the three hours of observation after resuscitation. At the end of the three hour observation period, shed blood is returned, catheters and probes are disconnected, and the animal is returned to the animal facility for seven more days in the metabolic cage with full food and water and daily blood sampling and urine collection. Finally, seven days after hemorrhage and resuscitation the animals are terminally anesthetized, two 20 minute urine collections are obtained from the right ureter, and the animal is euthanized and necropsied.

PILOT STUDY: Four swine were water deprived and hemorrhaged according to the protocol, and their hemodynamic responses and biochemical changes averaged to determine if dehydration and shock were achieved. The swine were randomly assigned to four different resuscitation solutions: 1) Ringer's lactate, 75 ml/kg; 2) 7% human albumin in Ringer's acetate, 25 ml/kg; 3) 10% stroma-free human hemoglobin (SFH) in Ringer's acetate, 25 ml/kg; and 4) 10% human hemoglobin cross-linked between the alpha chains with bis-(3,5-dibromosalicyl) fumarate ($\alpha\alpha$Hb) in Ringer's acetate, 25 ml/kg. Both hemoglobin solutions were produced in our production facility[4]. They had endotoxin concentrations of 0.1 EU/ml for SFH and 1 EU/ml for $\alpha\alpha$Hb.

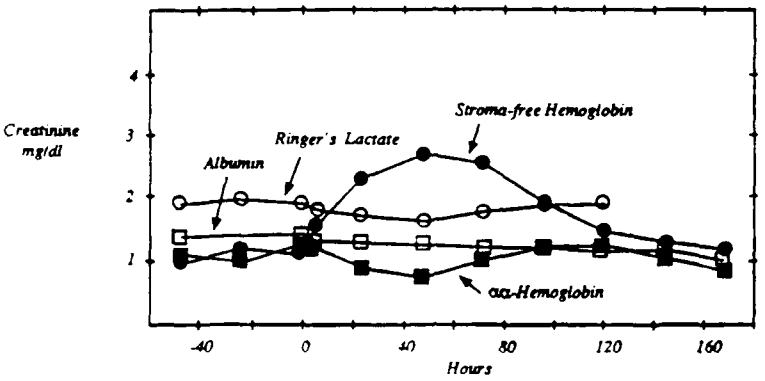

Figure: Plasma creatinine concentrations measured daily in the four swine in the pilot protocol from two days before hemorrhage and resuscitation to seven days afterward. Only the stroma-free hemoglobin treated pig had elevated concentrations after resuscitation.

RESULTS: Withholding drinking water for 48 hours achieved significant dehydration in the four swine with a 7% loss of body weight, plasma sodium increased from 143 to 152 mEq/l, and hemoconcentration occurred with a rise in hematocrit from 27.0 to 29.5. This is consistent with a loss of 12% of total body water. Hemorrhage produced a decrease in mean blood pressure from 93 to 44 torr and a concomitant decrease in cardiac output of 40%. With the loss of intravascular hydrodynamic pressure, the colloid osmotic pressure of the plasma is relatively unopposed, causing interstitial fluid to move into the intravascular space and dilute the remaining blood. Hematocrit decreased 21% over the hour of hemorrhage, and oxygen transport, the product of cardiac output and oxygen carrying capacity, declined 53%. As a result of the decrease in oxygen transport the plasma lactate rose from 9.2 to 113 mg/dl.

All four solutions successfully resuscitated the swine, raising blood pressure and cardiac output and allowing return of the plasma lactate to normal levels in two hours. The hemoglobin solutions, both SFH and αα-Hb, caused marked elevations in blood pressure which persisted for the three hours of measurement.

In the week of metabolic observation that followed the hemorrhage and resuscitation, the swine quickly reestablished normal plasma electrolyte concentrations and body weight gain. The plasma creatinine of the SFH-treated pig rose to 3 mg/dl and returned to normal on the fifth day (see Figure). There were no other significant metabolic changes seen in followup.

The Ringer's lactate treated animal died on the fifth day of metabolic follow-up of Staphylococcal pneumonia from catheter related sepsis. To prevent this complication as much as possible in other animals, subsequent animals have been given daily cephalosporin antibiotics. There were no other significant findings at necropsy or histomorphic examination of the tissues of any of the animals.

CONCLUSIONS: Water restricted and hemorrhaged swine appear to be a successful animal model of battlefield dehydration and shock. Resuscitation is effective with test and control solutions making treated animals available for observation of toxicity. The model demonstrates the expected renal toxicity of uncross-linked hemoglobin in resuscitation doses. Because of the complexity of the model and the large commitment of resources that it requires, the pilot study results are gratifying in suggesting that the model is efficient and effective.

The model also reveals a hypertensive effect with both hemoglobin solutions. Hypertension has been reported when hemoglobin solutions have been administered to animals[6] and humans[5], but the question of cause is confused by the possibility of contamination of the treatment solutions by toxic substances. The question can only be answered by further testing of materials of unquestioned purity.

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The experimental studies of the authors described in this report were reviewed and approved by the Institutional Review Committee/Animal Care and Use Committee at Letterman Army Institute of Research. The manuscript was peer reviewed for compliance prior to submission for publication. In conducting the research described here, the authors adhered to the "Guide for the Care and Use of Laboratory Animals," DHEW Publication (NIH) 85-23.

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